

WHAT IS CLAIMED IS:

1. A method for detecting at least one single stranded or double stranded nucleobase-containing target sequence in a fluid medium, said method comprising:

5 adding to said fluid medium antisense probes capable of forming hybridization complexes with said at least one nucleobase-containing target sequence, wherein said antisense probes comprise a backbone having a charge that is less negative than a comparable phosphodiester backbone;

10 separating unhybridized antisense probes from said hybridization complexes to form a test medium;

15 irradiating said test medium with a laser beam having a wavelength which excites fluorescent markers in said hybridization complexes and causes said fluorescent markers to emit fluorescent light;

measuring an intensity of said emitted fluorescent light; and

20 comparing said measured intensity with a reference intensity to detect whether said fluid medium contains said at least one target sequence,

wherein <sup>inverse</sup> ~~an inverse of~~ said measured intensity is ~~inversely~~ proportional to a number of base mismatches between said at least one nucleobase-containing target sequence and said antisense probes, over a range inclusive of 0 base mismatches through at least

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3 base mismatches, and wherein said method other than said separating step is entirely conducted without binding said antisense probes, said at least one nucleobase-containing target sequence or said hybridization complex to a solid support or gel.

2. The method of claim 1, wherein said backbone comprises at least one non-ionic linking group between a 5'carbon and a 3'carbon of adjacent sugars.

3. The method of claim 2, wherein said at least one non-ionic linking group is a methylphosphonate group.

4. The method of claim 1, wherein said backbone comprises a modified phosphodiester backbone having at least about 10% of its phosphate groups replaced with non-ionic groups.

5. The method of claim 4, wherein said non-ionic groups are methylphosphonate groups.

6. The method of claim 1, wherein said backbone comprises a modified phosphodiester backbone having at least about 20% and not more than about 80% of its phosphate groups replaced with non-ionic groups.

7. The method of claim 6, wherein said non-ionic groups are methylphosphonate groups.

8. The method of claim 1, wherein said backbone comprises a modified phosphodiester backbone having about 25% of its phosphate groups replaced with non-ionic groups.

9. The method of claim 8, wherein said non-ionic groups are methylphosphonate groups.

10. The method of claim 1, wherein said backbone comprises a modified phosphodiester backbone having about 50% of its phosphate groups replaced with non-ionic groups.

11. The method of claim 10, wherein said non-ionic groups are methylphosphonate groups.

12. The method of claim 1, wherein said backbone comprises at least one peptide segment and at least one phosphodiester segment.

13. The method of claim 1, wherein said backbone comprises at least one peptide segment and at least one methylphosphonate segment.

14. A method for detecting at least one single stranded or double stranded nucleobase-containing target sequence in a fluid medium, said method comprising:

adding to said fluid medium antisense probes capable of forming a hybridization complex with said at least one nucleobase-containing target sequence, wherein said antisense probes comprise a backbone having a charge that is less negative than a comparable phosphodiester backbone;

irradiating said fluid medium with a laser beam having a wavelength which excites fluorescent markers in said hybridization

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complex and causes said fluorescent markers to emit fluorescent light;

measuring an intensity of said emitted fluorescent light;  
and

5 comparing said measured intensity with a reference intensity to detect whether said fluid medium contains said at least one target sequence,

10 wherein said measured intensity is proportional to a number of base mismatches between said at least one nucleobase-containing target sequence and said antisense probes, over a range inclusive of 0 base mismatches through at least 3 base mismatches, and wherein said method is conducted without separating unhybridized probes from hybridization complexes prior to said signal detecting, and without providing a signal quenching agent on  
15 said antisense probes or on said at least one nucleobase-containing target sequence.

15. The method of claim 14, wherein said measured intensity is inversely proportional to an amount of hybridization complexes in said fluid medium and proportional to an amount of said  
20 antisense probes unhybridized in said fluid medium.

16. The method of claim 14, wherein said backbone comprises at least one non-ionic linking group between a 5'carbon and a 3'carbon of adjacent sugars.

17. The method of claim 16, wherein said at least one non-ionic linking group is a methylphosphonate group.

18. The method of claim 14, wherein said backbone comprises a modified phosphodiester backbone having at least about 10% of its phosphate groups replaced with non-ionic groups.

19. The method of claim 18, wherein said non-ionic groups are methylphosphonate groups.

20. The method of claim 14, wherein said backbone comprises a modified phosphodiester backbone having at least about 20% and not more than about 80% of its phosphate groups replaced with non-ionic groups.

21. The method of claim 20, wherein said non-ionic groups are methylphosphonate groups.

22. The method of claim 14, wherein said backbone comprises a modified phosphodiester backbone having about 25% of its phosphate groups replaced with non-ionic groups.

23. The method of claim 22, wherein said non-ionic groups are methylphosphonate groups.

24. The method of claim 14, wherein said backbone comprises a modified phosphodiester backbone having about 50% of its phosphate groups replaced with non-ionic groups.

25. The method of claim 24, wherein said non-ionic groups are methylphosphonate groups.

26. The method of claim 14, wherein said backbone comprises at least one peptide segment and at least one phosphodiester segment.

27. The method of claim 14, wherein said backbone comprises at least one peptide segment and at least one methylphosphonate segment.

28. The method of claim 1, wherein said at least one nucleobase-containing target sequence is a first segment within a folded nucleotide sequence, and said measured intensity is compared with a second measured intensity of a second segment within said folded nucleotide sequence to identify antisense probe accessible regions in said folded nucleotide sequence.

29. The method of claim 14, wherein said at least one nucleobase-containing target sequence is a first segment within a folded nucleotide sequence, and said measured intensity is compared with a second measured intensity of a second segment within said folded nucleotide sequence to identify antisense probe accessible regions in said folded nucleotide sequence.

30. The method of claim 1, wherein the number of base mismatches between said at least one nucleobase-containing target sequence and said antisense probes is determined.

31. The method of claim 14, wherein the number of base mismatches between said at least one nucleobase-containing target sequence and said antisense probes is determined.

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